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6-Phosphogluconolactonase deficiency, a hereditary erythrocyte enzyme deficiency: Possible interaction with glucose-6-phosphate dehydrogenase deficiency

(anemia/hemolysis)

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ABSTRACT **Partial deficiency of 6-phosphogluconolactonase (EC 3.1.1.31) of the erythrocytes was discovered as an autosomal dominant disorder. Hemolytic anemia occurred in an individual who had inherited both the gene for 6-phosphogluconolactonase deficiency and that for deficiency of a nonhemolytic variant of glucose-6-phosphate dehydrogenase (EC 1.1.1.49). It is proposed that the interaction of this hereditary erythrocyte abnormality with glucose-6-phosphate dehydrogenase deficiency may explain hemolysis in some other patients who have inherited polymorphic variants of glucose-6-phosphate dehydrogenase.**

6-Phosphogluconate is often regarded as the product of the glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) reaction. However, it has long been recognized that in reality the δ-lactone of 6-phosphogluconate is the initial reaction product (1–3) and that 6-phosphogluconate itself is formed only when the lactone is hydrolyzed.

$$\text{Glucose-6-}P + \text{NADP}^+ \rightarrow \text{6-phosphoglucono-}\delta\text{-lactone} + \text{NADPH} + \text{H}^+$$

$$\text{6-phosphoglucono-}\delta\text{-lactone} + \text{H}_2\text{O} \rightarrow \text{6-phosphogluconate}$$

$$\text{6-phosphogluconate} + \text{NADP}^+ \rightarrow \text{ribulose-5-}P + \text{NADPH} + \text{H}^+ + \text{CO}_2.$$

Described some 30 years ago (3), 6-phosphogluconolactonase (6PGL; EC 3.1.1.31) has been a much-neglected enzyme. This has been the case because the substrate of the enzyme is very unstable, with a half-life that has been estimated under different conditions to be 1.5 (2), 6, and 16 min (4). Thus it has often been assumed that such enzymatic activity as exists must be superfluous; in any case, from a practical point of view, its assay has been considered to be quite difficult to perform. However, recently Bauer *et al.* (4) showed that the γ-lactone, which is easier to prepare and a little more stable, would serve as an artificial substrate for 6PGL. We have now devised a facile method of preparing this substrate and have been able to study the activity of this enzyme in the erythrocytes of patients with hemolytic anemia. We report the discovery of a family with a partial deficiency of 6PGL and provide evidence that this enzyme deficiency may interact with G6PD deficiency to cause moderately severe hemolytic anemia.

METHODS

The γ-lactone of 6-phosphogluconic acid was synthesized by a modification of the method described by Bauer *et al.* (4). 6-Phosphogluconic acid was dried at 55°C under a stream of nitrogen. It was mixed with ethylene glycol monomethyl ether and again dried under nitrogen. It was then placed into a vacuum desiccator over silica gel, dissolved in dimethyl sulfoxide, and stored in liquid nitrogen. Details of the preparation of this substrate will be published elsewhere (5).

6PGL activity was assayed spectrophotometrically by measuring the formation of 6-phosphogluconate from its γ-lactone, following the reduction of NADP to NADPH in the presence of 6-phosphogluconate dehydrogenase (EC 1.1.1.44). Assays were carried out at 25°C in a system containing 1 mM triethanolamine buffer at pH 7.0, 0.6 mM NADP, 0.15–0.2 unit of 6-phosphogluconate dehydrogenase per ml, and 0.2 mM 6-phosphoglucono-γ-lactone. An initial rapid increase in absorbance due to 6-phosphogluconate contaminating the substrate was allowed to proceed to completion, requiring 1 or 2 min. After this, a 1:20 hemolysate (6) was added and the rate of change of absorbance at 340 nm was recorded. A blank rate was determined with water in place of hemolysate. A unit is defined as the amount of 6PGL leading to reduction of 1 μmol of NADP per min.

RESULTS

We performed 6PGL assays on the erythrocytes of 158 unrelated individuals; 132 of these were normal blood donors and 26 were patients with hemolytic anemia. Fig. 1 shows the distribution of enzyme activity observed in these subjects. One patient, a 14-month-old girl with hemolytic anemia, was found to have erythrocyte 6PGL activity well below that of all other subjects. Assay of the enzyme activity of the erythrocytes of all available family members confirmed the hereditary nature of the enzyme deficiency (Fig. 2). The pattern of inheritance was clearly that of a single autosomal gene coding for little or no enzyme activity, three generations being affected. The residual enzyme was found to have normal electrophoretic mobility on Cellogel at pH 6.5, normal thermal stability, and normal substrate affinity.

The activities of a panel of 22 erythrocyte enzymes (6) were measured on the erythrocytes of the child with hemolytic anemia, and she was found to have 8.20 international units of G6PD per g of Hb on one occasion and 6.58 international units/g of Hb on another (normal value mean ± SD = 12.1 ± 2.1 international units/g of Hb). The activities of other age-related enzymes such as pyruvate kinase, pyrimidine-5′-nucleotidase, and glutamic oxaloacetic transaminase were all increased. Clearly she was a heterozygote for G6PD deficiency. In addition, her erythrocytes were microcytic, and globin chain synthetic studies (7) established a diagnosis of α-thalassemia minor. The mother and grandmother, of Swedish–English ancestry, were also both deficient in 6PGL and had normal G6PD activity, as did one aunt. A second

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Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; 6PGL, 6-phosphogluconolactonase.

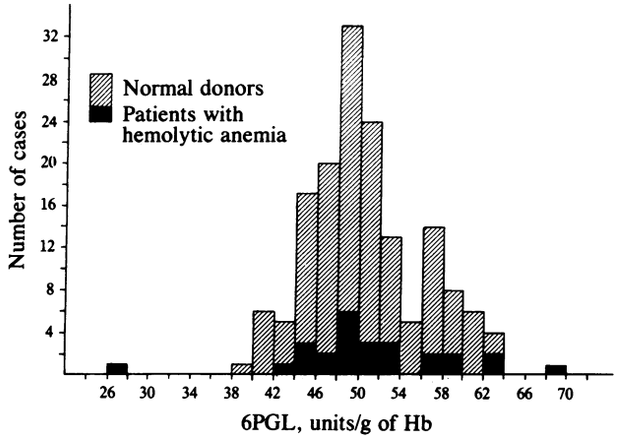

FIG. 1. Distribution of 6PGL activity of the erythrocytes of 132 normal subjects and 26 patients with hemolytic anemia.

aunt had normal erythrocyte 6PGL and G6PD levels. The father of the child, a Saudi Arabian, was said to be in good health but was not currently available for examination. The record of a blood count done some 2 years previously revealed that his hemoglobin level was normal and that microcytosis was present, presumably as a result of α-thalassemia trait. Since Saudi Arabia is an area in which the prevalence of G6PD deficiency is one of the highest in the world (8–10), it is to be presumed that the child had inherited a gene for G6PD deficiency from him.

DISCUSSION

Polymorphic variants such as G6PD Mediterranean do not cause hemolytic anemia in the vast majority of those who inherit them (8). Occasionally persons with such variants do experience chronic hemolysis even when other family members inheriting the same variant do not. We previously suggested that the effects of an unidentified autosomal gene might be exerting an effect in some of the G6PD-deficient individuals to cause marked shortening of the erythrocyte life span (11). The family we have described here suggests that the gene for 6PGL deficiency may play such a role. Half-normal levels of 6PGL did not by themselves cause impairment of erythrocyte survival, yet we observed chronic hemolytic anemia for which no other explanation was evident in the single individual who inherited both genes.

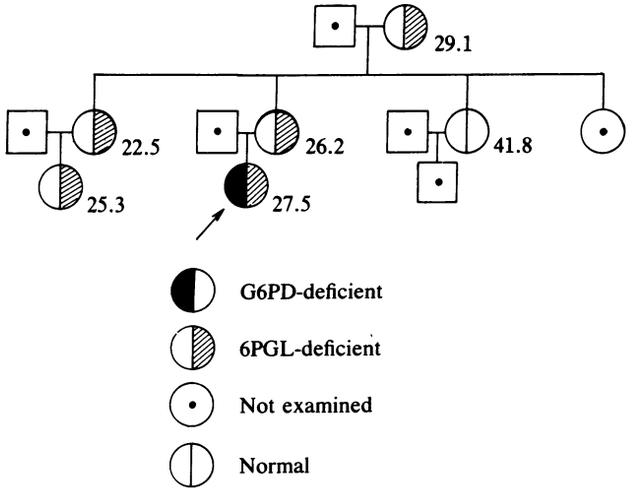

FIG. 2. Pedigree of a family with partial 6PGL deficiency. Numbers are 6PGL activities; normal value is 50.5 ± 6.1 units/g of Hb. The index case (↗) also exhibits G6PD deficiency and has hemolytic anemia.

Is it reasonable to speculate that half-normal levels of 6PGL would place erythrocytes at a metabolic disadvantage? Would not the hydrolysis of 6-phosphogluconolactone catalyzed by the residual enzyme be sufficient to serve the metabolic needs of the G6PD-deficient erythrocyte? The answer to this question is not yet entirely obvious. Normal erythrocytes contain sufficient G6PD to oxidize about 2.8 μmol of glucose-6-*P* per min per ml in the hexose monophosphate pathway (6). The unstimulated metabolic flow through the hexose monophosphate pathway, of which the G6PD reaction is the first step, is only about 1 nmol per min per ml (12, 13), or about 0.03% of the maximal activity of G6PD. In the Mediterranean type of G6PD deficiency the residual enzyme activity ranges from only 0.03% to 0.18% of normal (14). Erythrocyte life span is usually normal, or nearly so, in this type of deficiency. Apparently compensation is achieved by the greater-than-normal resistance of the mutant enzyme to inhibition by NADPH (15), its greater affinity for both NADP and glucose-6-*P*, and a marked downward shift of the NADPH/NADP ratio from a normal of 0.9825 to 0.2450 in the Mediterranean type of deficiency (16). But the extent that such compensation may occur is limited by the total amount of enzyme present, and even a modest increase in the level of one of the reaction products may produce sufficient impairment of flow through the G6PD reaction so that compensation is no longer possible. At any given rate of metabolic flow, reducing the level of 6PGL to one-half of normal will result in a doubling of the steady-state level of the lactone in the erythrocyte. In erythrocytes that are otherwise normal, this does not appear to impair function. In erythrocytes in which the available amount of G6PD is already borderline at best, the result may well be decompensation of the precarious metabolic state of these cells.

Such considerations make it seem to us quite possible that in the kindred we have studied a cause-and-effect relationship exists between the presence of partial lactonase deficiency and hemolysis in the G6PD-deficient proband. It may well be that other consequences of G6PD deficiency, such as sensitivity to the hemolytic effect of the fava bean, which are limited to only some G6PD-deficient individuals, may well also prove to be due to deficiency or malfunction of the 6PGL reaction. Alternatively, one must consider the possibility that the association of hemolysis and 6PGL deficiency in the G6PD-deficient patient was fortuitous. Detection of more cases of deficiency will be required to clearly define the role of 6PGL in erythrocyte physiology.

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